

Integration of sample cleanup methods into analytical supercritical fluid extraction

ANALYTICAL SUPERCRITICAL fluid technology (ASFT) is a sample preparation technique that continues to evolve in sophistication as it is applied to a diverse number of sample types and matrices. Initial attempts to utilize ASFT for sample preparation were largely devoted to employing the technology in the extraction mode, better known as supercritical fluid extraction (SFE). SFE has been widely applied in food and agricultural analysis,^{1,2} to environmental samples,^{3,4} and more recently for pharmaceutical assays.⁵ A number of collaborated supercritical fluid-based methods exist,^{6,7} while others are currently being verified.

With increasing utilization, ASFT-based techniques are becoming more complex, involving other aspects of sample preparation beyond the simple SFE mode. Of particular note is the integration of extract or sample cleanup along with the basic SFE step, to yield a simplified extract composition that is directly amenable for analysis. Over

the past five years, the author's laboratory has been a key contributor in integrating the cleanup mode with SFE, particularly to yield lipid-free extracts from foodstuffs that are amenable to established GC or HPLC assays. The basic concepts involved in many of these integrating cleanup methods have their origin in chromatography, ranging from the application of the solubility parameter theory⁸ to normal-phase chromatographic concepts.^{9,10} It is particularly appropriate in this article that the author acknowledge Prof. Barry Karger, who was his Ph.D. mentor at Northeastern University, for providing some of the knowledge in separation science that led to the development of these various supercritical fluid-based cleanup options.

Techniques for simplifying supercritical fluid-derived extracts

As shown in Table 1, there are a number of ways to simplify a supercritical fluid-derived extract. These include, of course, varying the pressure, temperature, and time of extraction to yield an extract containing the target analytes of interest and a reduced number of coextractives (if there are any). More specific options, such as changing the identity of the extraction fluid,¹¹ have achieved some success, while fractionation according to specific solute threshold pressures¹² is also limited in applicability. Such relatively simple approaches do not always work well since the resolving power of neat SFE is limited; consequently, coupling analytical SFE with adsorbents is frequently prac-

Table 1
Techniques for simplifying supercritical fluid extracts

Extraction variables:	Pressure Temperature Time
Postextraction solvent partition	
In situ adsorption:	Inverse SFE Matrix solid-phase dispersion
Chromatography:	Adsorption mode Size exclusion mode Complexation mode
Postextraction trapping	Solid-phase extraction Sorbent trapping
Coupled on-line methodology	Liquid chromatography Supercritical fluid chromatography

ticed. In situ adsorption methods involve adding the sorbent, usually after the sample to be extracted, to impart additional selectivity over that which can be achieved by changing the variables that control SFE. Variations in this theme include inverse SFE, which will be described later, and a supercritical fluid form of matrix solid-phase dispersion.¹³

The use of minichromatographic columns or cartridges in series with the extraction cell has also been reported¹⁴ and has involved retention mechanisms well known to chromatographers: namely adsorption,¹⁵ size exclusion,¹⁶ and complexation.¹⁷ It is also possible to use these same mechanisms of retention after SFE has been enacted and the resultant extract decompressed onto a sorbent-filled trap. This postextraction trapping can include the use of traditional solid-phase extraction

Dr. King is Lead Scientist in the Food Quality & Safety Research, National Center for Agricultural Utilization Research, Agricultural Research Service/USDA, 1815 N. University St., Peoria, IL 61604, U.S.A.; tel.: 309-681-6203; fax: 309-681-6686; e-mail: kingjw@mail.ncaur.usda.gov. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

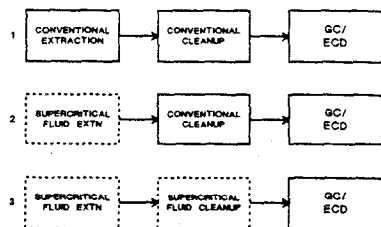


Figure 1 Integration of supercritical fluid extraction and cleanup methods into analytical methodology.

(SPE), or sorbent trapping as applied to more volatile extracted species.¹⁸ Direct coupling of analytical SFE with liquid or supercritical fluid chromatography has also been reported¹⁹ but not widely practiced for routine analysis. The advantage of all of these techniques relative to most liquid-based cleanup methods is the elimination of or substantial reduction in solvent use.

Sorbents that have been utilized to provide extract cleanup in SFE include many of the popular media employed by chromatographers: silica, alumina, bonded silicas, Tenax, polyurethane foams, sorbent disks, etc. Sorbents approximating those used in normal-phase chromatography have received the most use since analytes can be conveniently eluted using supercritical carbon dioxide (SC-CO₂). This is consistent with the low elutropic strength of SC-CO₂, even at higher pressures.²⁰

Adsorption chromatography coupled with SFE

Perhaps the simplest coupling of adsorption chromatography with analytical SFE is illustrated in *Figure 1*. Here we see a comparison of supercritical fluid analog with a conventional sorbent-based cleanup method (Method 1) for eliminating coextracted lipid interferences from targeted organochlorine pesticide residues. If one simply applies SFE as shown in Method 2, the target analytes can be successfully extracted,²¹ but a conventional cleanup technique, such as alumina or

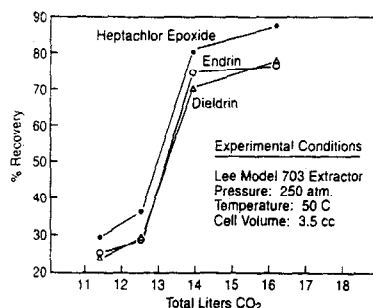


Figure 2 Percent pesticide recovery versus total carbon dioxide volume through extraction cell packed with alumina sorbent.

size exclusion, must be employed to separate the coextracted fat moieties from the pesticide analytes. However, Method 2 can be improved upon inserting an alumina sorbent bed after the extracted sample (Method 3), so that the fat is retained relative to the target pesticides using an appropriate pressure and temperature.²² As in normal adsorption chromatography-based cleanup systems, sorbent strength must be tempered by addition of water to the sorbent before SFE.

Use of the above normal-phase adsorption technique requires that several factors be assessed and controlled for the technique to work in the supercritical fluid mode. The analyte retention characteristics must be assessed as a function of the total quantity of supercritical fluid eluent passed through the sorbent bed to successfully capture the analytes. This is illustrated in *Figure 2*, where the breakthrough of three organochlorine pesticides from an alumina cleanup sorbent follows a classic sigmoidal frontal breakthrough curve for SC-CO₂ at 250 atm, and 50 C. This elution pattern, expressed in terms of total expanded volume of CO₂ through the sorbent bed, was accomplished using 1.8 g of alumina in a 3.5-cc extraction cell. In this case, approx. 0.2 g of sample was initially put on top of the alumina bed.

Recovery of analytes from the sorbent bed may be aided by the addition of a very small quantity of co-

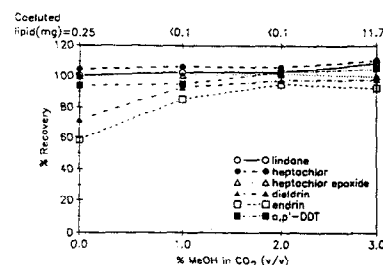


Figure 3 Effect of methanol addition on the supercritical carbon dioxide cleanup of a spiked lard extract.

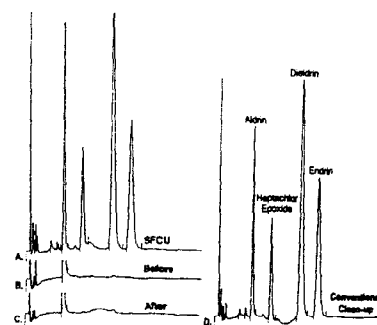


Figure 4 GC-ECD (electron capture detection) chromatograms of cleaned-up supercritical fluid extract from poultry adipose tissue (SFCU = supercritical fluid cleanup).

solvent to the SC-CO₂ as shown in *Figure 3*, for the elution of organochlorine pesticides from silica. Here, recovery of two of the organochlorine pesticides is substantially improved by the addition of 2.0% by volume of methanol in SC-CO₂. However, it should be noted that adding any additional quantity of methanol to the SC-CO₂ results in breakthrough of interfering lipid species, which is not desired. However, when optimized, adsorption chromatography coupled with SFE can produce results equivalent to those obtained via conventional liquid adsorption chromatography, as is nicely illustrated by the gas chromatographic/electron capture profiles in *Figure 4*.²²

Inverse SFE

Another useful example of integrating the sample cleanup step in SFE is the use of inverse SFE. The author initially demonstrated this

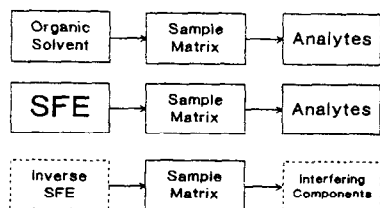


Figure 5 Comparison of inverse SFE with conventional SFE.

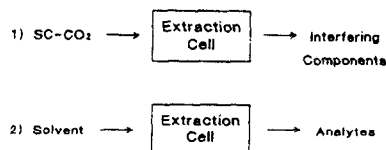


Figure 6 Operational sequence for inverse SFE.

concept several years ago²³ and coined the term inverse SFE for this technique, which is illustrated in Figure 5. As shown in the first two sequences in Figure 5, the addition of an adsorbent into the extraction sequence is normally utilized to yield a simplified extract containing the analytes of interest. In inverse SFE, the sorbent is added to the extraction, or in-line as a separate bed, to facilitate the removal of the interfering components from the subsequent assay. This is frequently done by using neat SC-CO₂ to remove the unwanted compounds, i.e., fats or other nonpolar compounds, from the sorbent bed. Then, as noted in Figure 6, a stronger solvent (perhaps a cosolvent in SC-CO₂) is used to displace the target analytes from the sorbent bed.

A colorful example of this process is shown by the sequence in Figure 7. Here, a chromophoric target analyte, LGV, has been added at a very high level (100 ppm) to poultry fat for illustrative purposes (Figure 7a). The sample is then mixed with an extraction enhancer²⁴ called Hydromatrix (Varian Corp., Harbor City, CA) (contained in the center vial in Figure 7b) to produce the speckled mixture on the far right. This is then placed in the extraction vessel containing additional Hydromatrix as the adsorbent, and SFE

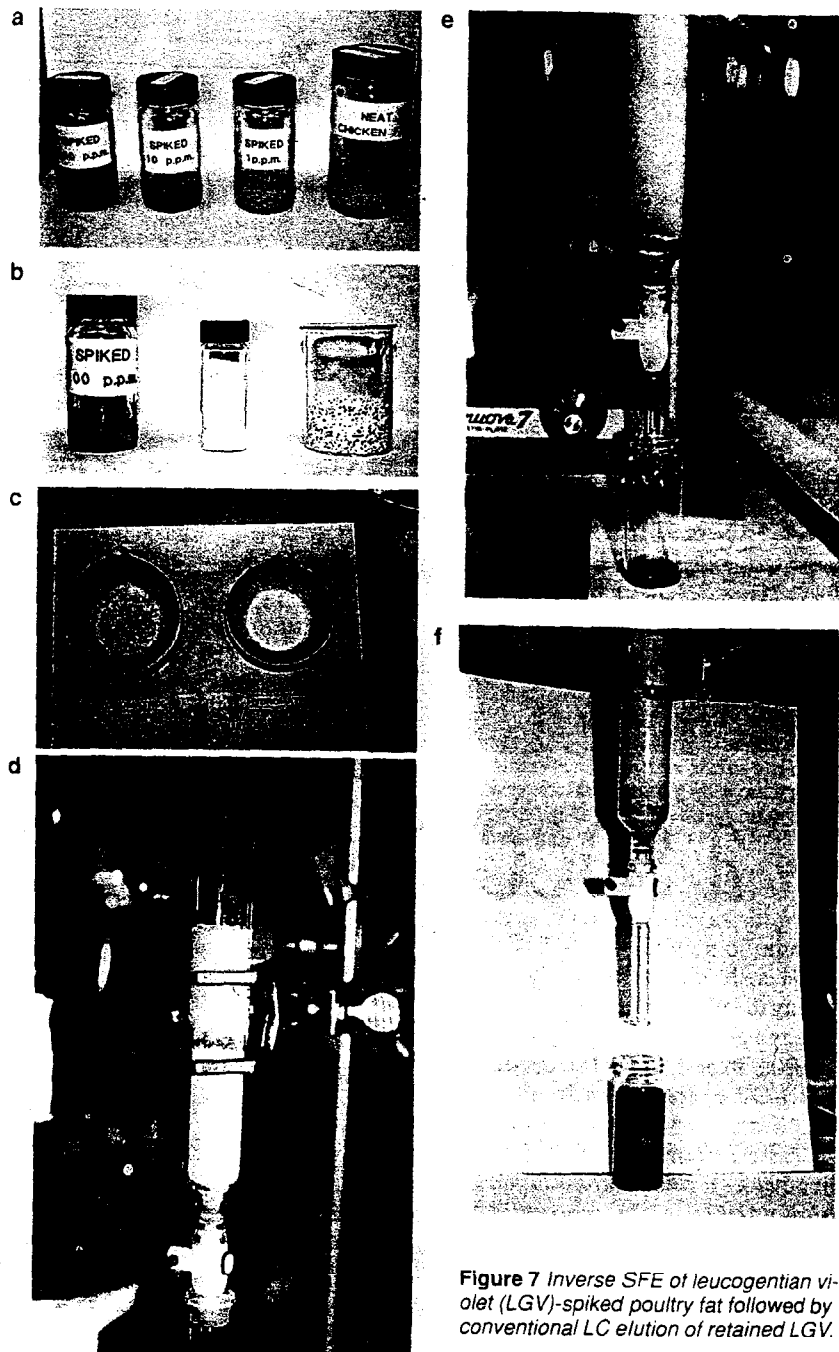


Figure 7 Inverse SFE of leucogentian violet (LGV)-spiked poultry fat followed by conventional LC elution of retained LGV.

commenced. After the interfering lipid contaminants have been removed via SFE, the Hydromatrix is carefully removed from the high-pressure extraction cell to allow the elution process to be observed (normally this can be done in-line with the extraction cell). As noted in Figure 7c, the target analyte, LGV, has been retained on the supercritical

fluid-extracted sorbent (the Hydromatrix). This SC-CO₂-extracted sorbent is then placed in a glass chromatographic column for illustrative purposes and eluted with ethanol under gravity (sequence in Figures 7d-f), to yield the desired analyte, lipid-free.

To further verify the above fractionation mechanism at a lower ana-

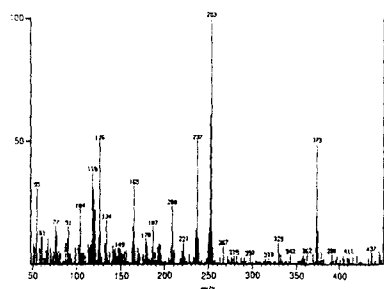


Figure 8 Electron impact mass spectrum of LGV from SFC-MS.

lyte level (5 ppm) and provide a scheme for isolating the eluted fractions, eight experiments were conducted under slightly different conditions, as reported in Table 2. Here, a trap was employed downstream from the extraction cell, packed in some cases with 4 g of silica, to help trace the elution of the LGV throughout the system. In the first three experiments, glass wool was employed in the cell rather than Hydromatrix to see if the Hydromatrix would retain the LGV under certain extraction conditions (experiments 4–8). An empty vessel (glass round-bottom flask) was also placed downstream from the trap to see if any LGV eluted off the silica-filled trap, as well as to collect any lipid extracted by the SC-CO₂. As shown by the first three experiments in Table 2, glass wool in the extraction cell was not sufficient to retain the LGV moiety at pressures cited and after passage of variable volumes of CO₂. Most of the LGV was found on the silica trap that was downstream from the Hydromatrix-packed extraction cell. However, by packing the cell with 12 g of Hydromatrix, with or without silica in the trapping assembly, the target analyte, LGV, was found to be retained in the extraction cell using several combinations of extraction pressures and volumes of extraction fluid. Therefore, it is feasible to obtain a lipid-free extract via inverse SFE.

Mass spectrometry coupled with capillary SFC was used to verify the identity of the analyte after extraction under the above conditions indicated in Table 2. The electron im-

Table 2

Extraction/fractionation of LGV from poultry fat							
Run no.	Substrate support	Silica trap (g)	Extraction pressure (psi)	CO ₂ (L)	%Recovery of LGV		
					Extraction cell	Trap	Receiver
1	Gl. wool	4	10,000	250	0	65	0
2	Gl. wool	4	5000	250	30	70	0
3	Gl. wool	4	10,000	150	8	88	0
4	12H	4	10,000	250	90	0	0
5	12H	4	5000	250	90	0	0
6	12H	NA	10,000	200	90	NA	7
7	15H	NA	10,000	150	90	NA	7
8	15H	NA	10,000	150	95	NA	0

LGV concentration in fat = 5 ppm; sample size = 5.0 g; extraction temp = 40 °C; xH = grams of Hydromatrix; NA = not applicable; Gl. wool = glass wool; extraction pressures given in psi.

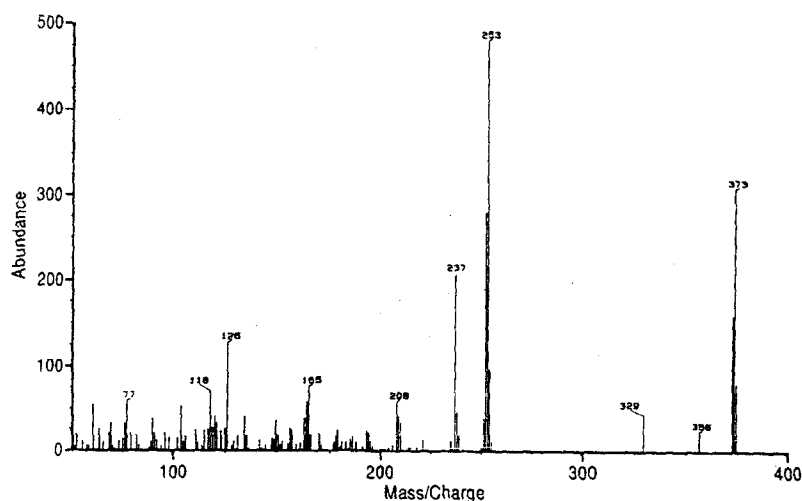


Figure 9 Electron impact mass spectrum of LGV from particle beam LC-MS.

pact mass spectra obtained by capillary SFC/MS analysis on the inverse SFE-defatted extract is shown in Figure 8. This matched well with the electron impact mass spectrum (Figure 9) obtained using particle beam LC-MS. The observed fragmentation pattern and principal mass/charge peaks are consistent with the proposed LGV fragmentation shown in Figure 10, including the molecular ion at $m/e = 373$ amu.

Alternative fluids

Other fluids besides SC-CO₂ have been used sparingly in ASFT. This is partly because they offer limited advantage over SC-CO₂,

they have some undesirable property associated with their use (e.g., N₂O), or their cost is prohibitive. However, for simplifying our resultant extracts, we have found fluorocarbon, HCF₃, to have some utility due to its low propensity for nonpolar compounds (i.e., lipids).²⁵ SFE of incurred organochlorine pesticide residues in poultry fat distributed in the extraction cell on a glass bead support showed that HCF₃ extracted 100-fold less fat than SC-CO₂ under corresponding conditions (250 atm, 50 °C, 50 mL of HCF₃ or CO₂).²⁶ The HCF₃-derived extract could then be diluted and directly injected for GC-ECD analysis of the organochlorine pesticides. The resultant

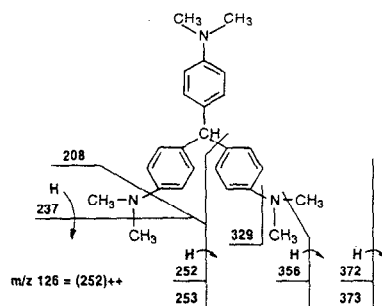


Figure 10 Electron impact mass spectrometry decomposition mechanism for LGV.

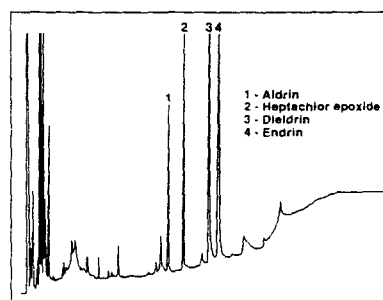


Figure 11 GC-ECD chromatogram on incurred pesticides in poultry fat using SC-HCF₃.

chromatogram is shown in *Figure 11*, where the three organochlorine pesticides in the adipose tissue as well as the internal standard (aldrin) can be readily detected at the 1–3 ppm level, relatively free of any interferences. This is indicative of the superior discriminating power of the HCF₃ relative to lipid coextractives.

A rationale for this result can be seen in *Figure 12*, where the solubility parameters of the two fluids have been plotted as a function of pressure, relative to the solubility parameter of a major fat constituent, a triglyceride. The pressure-based solubility parameters of the two fluids were calculated according to the method proposed by Giddings,²⁷ which the authors have found useful in understanding solute-solvent interactions in SFE.²⁸ Note that at lower pressures, the solubility parameter of SC-HCF₃ is greater than that of SC-CO₂, but as more pressure is applied, the opposite is true, indicating that SC-CO₂ becomes the better solvent for a triglyceride molecule than HCF₃. Therefore, it is not

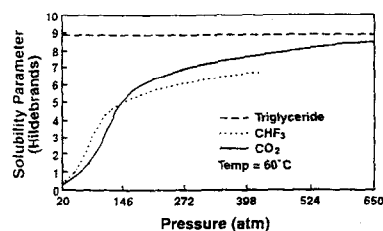


Figure 12 Solubility parameters of SC-CO₂ and HCF₃ as a function of extraction pressure.

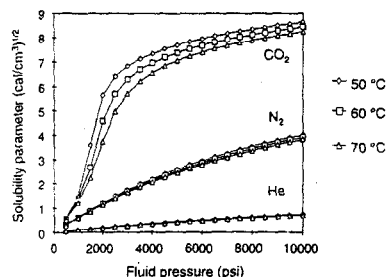


Figure 13 Solubility parameters of SC-CO₂, SC-N₂, and SC-He as a function of pressure and temperature.

surprising that an increased lipid solubility is recorded in SC-CO₂ relative to HCF₃. This approach has been used to particular advantage by Taylor and co-workers for the SFE of specific pesticide and drug moieties.^{29,30}

Another alternative fluid approach that has been found to be effective in fractionating lipid moieties from target analytes is the use of binary supercritical fluid mixtures. In this case, one uses a fluid that has a considerably lower critical temperature relative to the principal solvating fluid (i.e., SC-CO₂), but at an extraction temperature in which both gases can be regarded in their supercritical state. This type of binary fluid mixture has less solvating power than that possessed by the neat fluid with the higher critical temperature,³¹ but the binary fluid mixture does have sufficient solvating power to selectively extract trace levels of target analytes from lipid-rich matrices.³²

This can be better understood by invoking the solubility parameter concept, as shown in *Figure 13*, where the solubility parameters for

carbon dioxide, nitrogen, and helium have been plotted as a function of pressure. The solubility parameter in this case for SC-CO₂ can become quite large with increasing pressure; however, its discriminating power with respect to target analytes such as pesticides in fatty food matrices is limited. Reduction of the applied pressure on SC-CO₂ will reduce the amount of lipid matter extracted, but analyte recoveries are also reduced. As noted in *Figure 12*, the variation in nitrogen's solubility parameter as a function of pressure is substantially less; therefore, it was surmised based on evidence in the literature³¹ that mixtures of CO₂ and N₂ might provide sufficient solvation for extracting trace analytes while substantially reducing the extraction of lipid material.

Proof of this is shown in *Table 3*, where the amount of pesticide recovered along with the quantity of lipid coextracted, as a function of fluid composition at 10,000 psi and 70 °C, is noted. Both pure CO₂ and a 95 mol% CO₂/5 mol% N₂ extract 3.8 and 1.82 g of lipid under the above conditions. A composition of 20 mol% CO₂/80 mol% N₂ extracts approximately zero fat, but as noted in *Table 3*, the pesticide recoveries are very low. Using an intermediate composition of carbon dioxide with nitrogen (75 mol% CO₂/25 mol% N₂) reduces the coextracted lipids to 110 mg, while yielding 70% recoveries of the target analytes. Further optimization of this method (8000 psi, 60 °C, 70 mol% CO₂/30 mol% N₂) has permitted even larger recoveries of organochlorine pesticides while reducing coextracted fat to the 12-mg level.³²

Conclusion

In this brief review the author has tried to provide illustrative examples of how sample cleanup methods can be integrated into supercritical fluid-based extraction systems. Obviously, more than one method can yield the same result, and implementation depends on what approach is consistent with the analyst's facilities and sam-

Quick! Scan!

The SPECTRONIC GENESYS™ 2 UV/Vis Spectrophotometer Sets The Standard For Productivity, Performance And Value.

Obtaining high-quality, precise results time after time is easy with the SPECTRONIC GENESYS™ 2 UV/Vis spectrophotometer.

- 2 nm bandpass
- Advanced scanning capabilities
- Wide variety of preprogrammed tests
- Built-in automatic, 8-position cell holder
- Full-color screen

Discover the many advantages of the SPECTRONIC GENESYS™ 2 UV/Vis spectrophotometer for yourself.

820 Linden Avenue • Rochester, NY 14625
800-654-9955 • 716-248-4000
Fax: 716-248-4014
URL: <http://www.spectronic.com>
Email: info@spectronic.com
An ISO 9001 Company

AD33-250

A subsidiary of Thermo Optics, a Thermo Instrument Systems company.

Circle Reader Service No. 338

SAMPLE CLEANUP METHODS *continued*

Table 3

Pesticide recoveries and lipid extracted from poultry fat as a function of fluid composition

	Fluid composition (mol%)			
	Pure CO ₂	95% CO ₂ /5% N ₂	75% CO ₂ /25% N ₂	20% CO ₂ /80% N ₂
Lipid extracted (mg)	3800	1820	110	0
	Pesticide recovery %			
Heptachlor epoxide	100	100	70	6
Dieldrin	100	100	70	11
Endrin	100	100	65	9

Extraction pressure = 10,000 psi; extraction temp = 70 °C; total mass CO₂ = 90 g.

ple matrix. The fractionation effects observed are highly dependent on the solvent power of the compressed fluid and its interaction with the sample matrix, and sorbent, if one is utilized. Such systems are analogous to modifications that are made in LC to control retention and resolution, and further confirm the seminal principles that govern separation science.³³

References

1. Lehotay SJ. Supercritical fluid extraction of pesticide residues in fruits and vegetables. *Sem Food Anal* 1996; 73-84.
2. Dean JR. Applications of supercritical fluids in food science. In: Dean JR, ed.

Applications of supercritical fluids in industrial analysis. London: Blackie Academic & Professional, 1993:130-58.

3. Hawthorne SB. Analytical scale supercritical fluid extraction. *Anal Chem* 1990; 62:633A-42A.
4. McNally MEP. Advances in environmental SFE. *Anal Chem* 1995; 67:308A-15A.
5. Taylor LT. Supercritical fluid extraction. New York: John Wiley & Sons, Inc., 1996; 136-47.
6. AOCS Official Method AM3-96—Oil in oilseeds, supercritical fluid extraction method. Official methods and recommended practices of the American Oil Chemists Society—4th ed., vol 2. Champaign, IL: American Oil Chemists Society, 1997.
7. Lesnik B. The evolution of SFE. *Environmental Lab* 1996; 8(1):12-5.
8. Keller RA, Karger BL, Snyder LR. In:

Stock R, Perry SG, eds. *Gas chromatography*—1970. London: Institute of Petroleum, 1971.

9. Snyder LR. Principles of adsorption chromatography. New York: Marcel Dekker, Inc., 1968.
10. King JW. Analytical supercritical fluid techniques and methodology: Conceptualization and reduction to practice. *J Assoc Off Anal Chem Int* 1998; 81:1-9.
11. Taylor LT. Supercritical fluid extraction. New York: John Wiley & Sons, Inc., 1996.
12. King JW. Fundamentals and applications of supercritical fluid extraction in chromatographic science. *J Chromatogr Sci* 1989; 27:355-64.
13. Hopper ML, King JW. Enhanced supercritical fluid carbon dioxide extraction of pesticides from foods using pelletized diatomaceous earth. *J Assoc Off Anal Chem* 1991; 74:661-6.
14. Stolker AAM, Sipoli Marques MA, Zonnitjes PW, Van Ginkel LA, Maxwell RJ. Supercritical fluid extraction of residues of veterinary drugs and growth-promoting agents from food and other biological matrices. *Sem Food Anal* 1996; 1:117-32.
15. Maxwell RJ, Lightfield AR, Stolker AAM. An SPE column-Teflon sleeve assembly for in-line retention during supercritical fluid extraction of analytes from biological matrices. *J High Res Chromatogr* 1995; 18:231-4.
16. Taylor SL, King JW, Favati F, Hopper ML. Hybrid solvent systems for extraction and chromatography. Abstracts—

- 7th International Symposium on SFC and SFE. Cincinnati, OH: Supercritical Conferences, Inc., Abstract L-25, 1996.
17. Taylor LT. Strategies for analytical SFE. *Anal Chem* 1995; 67:364A-70A.
 18. Snyder JM, King JW. Oilseed volatile analysis by supercritical fluid and thermal desorption methods. *J Am Oil Chem Soc* 1994; 71:261-5.
 19. Nam K, King JW. Coupled SFE/SFC/GC for the trace analysis of pesticide residues in fatty food samples. *J High Res Chromatogr* 1994; 17:577-82.
 20. Randall LG. In: Ahuja S, ed. *Ultra high resolution chromatography*. Washington, DC: American Chemical Society, 1984; 135-69.
 21. Snyder JM, King JW, Rowe LD, Wornner JA. Supercritical fluid extraction of poultry tissues containing incurred pesticide residues. *J Assoc Off Anal Chem* 1993; 76:888-92.
 22. France JE, King JW, Snyder JM. Supercritical fluid-based cleanup technique for the separation of organochlorine pesticides from fats. *J Agric Food Chem* 1991; 39:1871-4.
 23. King JW. Chromatographic concepts in supercritical fluid extraction. In: *Proceedings of the 2nd European Symposium on Analytical Supercritical Fluid Chromatography and Extraction*. Heidelberg: Huethig Verlag, 1993:236-7.
 24. Hopper ML, King JW. Supercritical fluid extraction enhancer. U.S. Patent 5,151,188, Sept 29, 1992.
 25. Stahl E, Quirin KW, Gerard D. Dense gases for extraction and refining. Heidelberg: Springer-Verlag, 1988:175-7.
 26. Taylor SL, King JW. Supercritical fluid extraction of organochlorine pesticides using trifluoromethane. Allentown, PA: Air Products publication no. 320-9431, 1994.
 27. Giddings JC, Myers MN, McLaren L, Keller RA. High pressure gas chromatography of nonvolatile species. *Science* 1968; 162:67-73.
 28. King JW. Supercritical fluid extraction of polymers and solvents: Utilization of the solubility parameter concept. *Proc Div Polym Mat Sci & Eng* 1984; 51:707-12.
 29. Khorassani MA, Taylor LT, Schweighardt FK. Development of a method for extraction of organochlorine pesticides from rendered chicken fat via supercritical fluoroform. *J Agric Food Chem* 1996; 44:3540-7.
 30. Khorassani MA, Taylor LT, Schweighardt FK. Comparison of supercritical CHF₃ and CO₂ and methanol-modified CHF₃ and CO₂ for the extraction of sulfonamides from chicken liver. *J Assoc Off Anal Chem Int* 1996; 79:1043-9.
 31. Brunner G. In: Marinsky JA, Marcus Y, eds. *Ion exchange and solvent extraction—vol 10*. New York: Marcel Dekker, Inc., 1988:132-4.
 32. King JW, Zhang Z. Selective extraction of pesticides from lipid-containing matrices using supercritical binary gas mixtures. *Anal Chem* 1998; 70. Accepted for publication.
 33. Karger BL, Snyder LR, Horvath C. *An introduction to separation science*. New York: John Wiley & Sons, Inc., 1973.

HPLC '98

Daniel W. Armstrong, Ph.D., Chairman

22nd International Symposium on High Performance Liquid Phase Separations and Related Techniques

May 2 - 8, 1998 • St Louis, Missouri, USA
<http://www.stlcdg.org/hplc98>



Readers are invited to attend HPLC'98, the largest international meeting and exhibit dedicated to all facets of separations science.

Attendees can spend the day with many of the world's leading experts in a thought-provoking, dynamic environment where key issues will be debated, emerging trends analyzed, and new solutions uncovered. Then, experience the sights and sounds of St. Louis—fabulous restaurants, riverboat casinos, exciting night life, jazz and blues music. Delegates will have access to sessions on practical laboratory and industrial applications, new developments in instrumentation and separation media, and state-of-the-art procedures in chromatography and capillary electrophoresis. Plenary lectures will provide surveys of the various high resolution liquid phase separations technologies.

Grand Lecturer

- Prof. George Whitesides—Analysis and defense against chemical and biological weapons

Plenary Speakers

- Prof. Richard Zare—Taking the plunge into nanochemistry
- Prof. Michael Gross—Mass spectrometry and HPLC/MS for protein structure and function: studies by H/D exchange
- Prof. Barry Karger—Recent advances in biochemical microseparations and mass spectral analysis
- Prof. George Whitesides—Use of capillary electrophoresis to study electrostatic effects in biochemistry
- Dr. William Hancock—New approaches to the integrated genome/proteome analysis: the use of separations methods with on-line MS for analysis of viral particles
- Prof. Stellan Hjertén—Capillary chromatography and electrochromatography on continuous beds

Lectures by world renown scientists will provide more focus on areas of recent innovation and more detail on the state-of-the-art, and show the direction in which the various branches are growing. Posters will constitute the primary vehicle for scientific communications. To round out the meeting, the program will include short courses on the latest, most popular areas of liquid-based separations; planned discussion sessions that explore, analyze and debate innovative, hot topics in-depth; an exhibition; and seminars on applications of the latest research techniques to real world problems.

We encourage you to participate and to submit an abstract.
Contact the Symposium Manager for details.

PLEASE DIRECT INQUIRIES TO:

Ms. Janet Cunningham, Symposium/Exhibit Manager
Barr Enterprises
P.O. Box 279, Walkersville, MD 21793 USA
Phone 301-898-3772 / Fax 301-898-5596
E-mail Janetbarr@aol.com